Research Highlights

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Local heating of discrete droplets

Efficient heating of sample is a key requirement for many biological and chemical reactions. In microfluidic applications, heating is typically achieved by using Peltier devices, thin film heaters, or, by placing the entire chip on a hot plate.

In their recent work, Ji-Ho Park *et al.* describe a method for precise local heating of water droplets by utilisation of magnetic porous Si microparticles.[1] The microparticles consist of a hydrophobic top layer and a hydrophilic bottom layer that is infused with Fe_3O_4 nanoparticles. These microparticles have unique properties for handling of tiny water droplets: First, they are amphiphilic, thus spontaneously assemble at the interface between two immiscible liquids, *e.g.* at the interface of a water droplet immersed in mineral oil. Second, they are magnetic and facilitate manipulation of the water droplets and generation of heat inside the droplets by means of magnetic fields that can be remotely actuated. Third, a photonic code is etched into the hydrophobic layer of the particles that enables identification of individual droplets by reflectivity spectroscopy.

A simple biochemical application, *i.e.* the hybridization/dehybridization of DNA, is demonstrated. Two different droplets are prepared. Each droplet type contains single strand oligonucleotides, with complementary sequences, that are labelled with the fluorescent dyes Cy3 and Cy5, respectively. The droplets are half-covered by magnetic particles with a spectral code that produce either one or three peaks in the reflectivity spectrum (D1 and D2, see Fig. 1). Since half of the droplet surface is free of the (non-transparent) particles, fluorescence from inside the droplets can be observed. Upon merging of two droplets using a magnet, the DNA strands hybridize and a fluorescence signal is observed that results from fluorescence resonance energy transfer (FRET) from Cy3 to Cy5. By application of an oscillating magnetic field, the temperature is raised to 57 °C, which causes melting of the DNA and hence, a loss of FRET signal is determined. This reaction of binding and melting of DNA is performed in a periodic manner with good reproducibility of the temperature increases/decreases. The duration of efficient heating is slow compared to other techniques (here: 25 s for a temperature change from 37 to 57 °C), but the authors state that the setup has not been optimised for rapid cycling so far. Since the level of heating is related to the amount of magnetic microparticles, a group of discrete droplets can be simultaneously heated to different temperatures using a single coil.

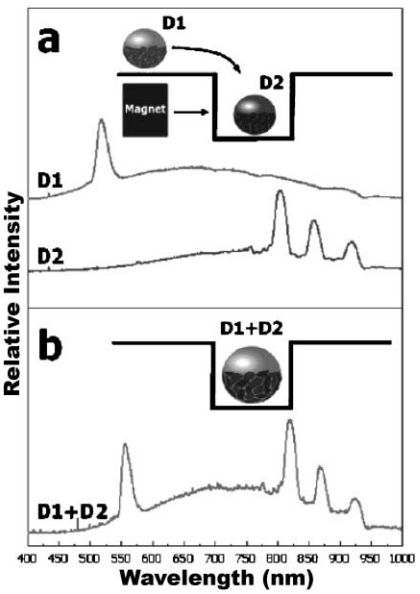

Fig. 1 Merging of two water droplets. The water droplets immersed in oil are surrounded by amphiphilic magnetic particles. The hydrophobic layer of the particles contains a photonic code that enables identification of droplets by reflectivity spectroscopy. (a) Reflectivity spectra of droplets that are half-covered by magnetic particles with the code D1 and D2, respectively. The droplets are trapped and merged in a shallow well by use of a magnet. (b) The reflectivity spectrum obtained after merging the droplets is a superposition of the two spectra shown in (a). (Reprinted with permission from Park *et al.*[1] Copyright 2006 American Chemical Society).

Artificial nose on a microchip

In vertebrates smells are sensed by the olfactory epithelium in the nasal cavity. The olfactory system can distinguish among hundreds of thousands of different odorants. This amazing performance of the "natural odorant sensor" relies on olfactory receptors (ORs), which are membrane proteins that belong to the large super-family of G protein coupled receptors. Each OR can recognise a range of odorants that share specific molecular features at very low concentration, and can discriminate slight discrepancies in odorant structure. This high specificity makes the ORs an excellent sensor, and the incorporation of natural olfactory receptors into sensor technology will provide the basis for a bioelectronic nose for tracking of volatile chemicals. Since odorants are omnipresent, *e.g* in food, drugs, respiration, explosives, and pollutants, the applications for rapid and non-invasive assessment of volatile odorants will be enormous in many fields.

Jasmina Minic Vidic *et al.* presented in a recent publication an approach towards such an artificial nose.[2] They developed a sensor for volatile odorants on chip-format utilizing an olfactory receptor and an appropriate G_α protein, which were co-expressed in yeast cells. The cells were mechanically disrupted. Nanosomes (nanometer-sized liposomes) were prepared and immobilized on a gold coated sensor chip *via* carboxylated dextran (Fig. 2). The nanosomes contain the ORs in their native membrane environment, and the G_α protein subunit is bound to the OR. Upon stimulation of an OR with an odorant ligand, the G_α subunit is activated and desorbed in the presence of GTP. The loss of the G_α subunit can be measured by surface plasmon resonance (SPR).

The sensing characteristics, *i.e.* the sensitivity and the selectivity, of the device were evaluated for a couple of odorants. The ORs in the nanosomes discriminate between odorant ligands and unrelated odorants, as in whole cells (Fig. 3). This demonstrates that the

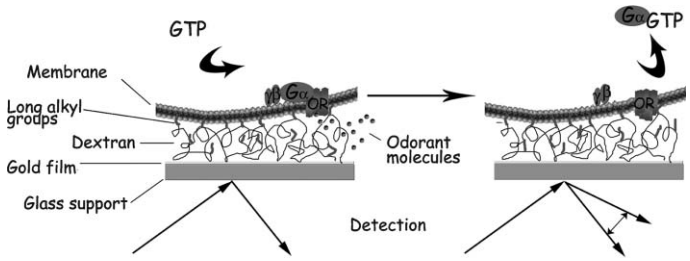

Fig. 2 Concept of the bioelectronic nose. An olfactory receptor is inserted in the lipid membrane of a nanosome. The nanosome is bound *via* dextran onto a sensor chip that is coated with a gold film. When a specific odorant molecule stimulates the olfactory receptor, it releases a subunit (G_α) in the presence of GTP. The desorption of the subunit can be observed by a change of the surface plasmon resonance response.

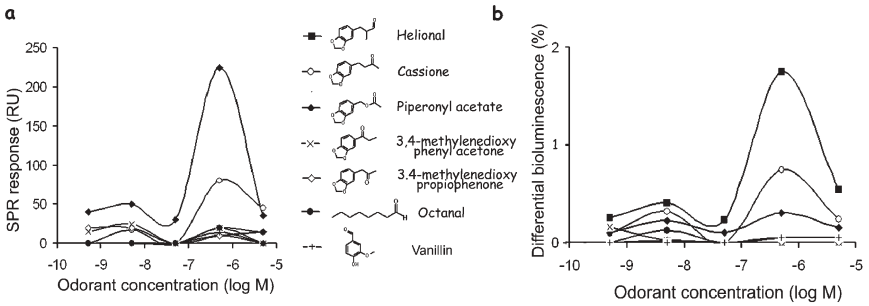

Fig. 3 Detection of odorants by the olfactory receptor (OR) cmyc-OR1740 inserted in (a) the nanosomes determined by surface plasmon resonance, and (b) in whole yeast cells determined using a bioluminescence assay. Sensitivity and specificity of the OR for various odorants is the same for the nanosomes and for the yeast cells, demonstrating that the activity of the OR in the bioelectronic nose (*i.e.* in the nanosomes) maintains its natural activity.

receptor activity is not hampered by the immobilization of the nanosomes. Furthermore, the device can be used repeatedly without loss of sensitivity, provided that the system is allowed to recover for a lag time of 30 min between successive stimulations. Besides the binding of odorants to the OR, the authors could directly study the coupling efficiency of the ORs with different G_α subunits without interference of other cellular pathways.

In conclusion, this chip-based sensor preserves the performance of ORs under operation conditions devoid of living cells. It requires only minute quantities of nanosomes, thus is valuable for any micro- and nanoscale sensor application.

On-demand patterning of protein matrixes

Matsuhiko Nishizawa and co-workers report a method to create a patterned surface within a microchannel by electrochemical means that enables site-specific immobilization of protein matrixes after the device is fully assembled.[3] The microfluidic device comprises a microelectrode array at the upper channel wall. The channels are flushed with a blocking agent (polyethyleneimine and heparin) rendering antibiofouling surfaces. Buffer solution containing KBr is then introduced into the microchannel, and the flow is completely stopped. Upon application of a potential pulse to the microelectrodes, Br_2, and subsequently hypobromous acid (HBrO), is generated at the electrodes, which diffuses across the channel and removes locally a portion of blocking agent. Thus, the surface in the proximity of the electrodes becomes adhesive to proteins. The area of reaction between the hydrobromous acid and the blocking agent can be controlled by the electrolysis period and the channel height.

Since several microelectrodes can be addressed separately, subsequent patterning of multiple proteins at distinct locations within the same channel is possible. This stepwise electrochemical treatment is demonstrated for immobilization of different antibodies. A sandwich immunoassay is performed that enables the simultaneous determination of two types of antigens, which could be beneficial for medical diagnosis.

Single-molecule analysis in multi-laminar flow

Studies on single-molecule level facilitate the investigation of functions and dynamics of single biomolecules in contrast to the time-and-space averaged observations in classical biochemical research. Besides the capability to image single molecules, it is crucial for comprehensive single-molecule studies to control the environmental conditions of a single molecule.

Sang Wook Lee *et al.* demonstrate in their work the controlled supply and removal of chemicals to an immobilized single molecule using multi-laminar streams generated in a microfluidic chip.[4] The concept of spatially controlled chemical delivery has already been employed for single cell treatment, where different streams can carry specific chemical stimulus to a cell, or a microdomain of a cell. In this work, the authors study the rotation of the enzyme F_1-ATPase. F_1-ATPase, a portion of ATP synthase, is a ubiquitous transmembrane enzyme that catalyzes the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). The hydrolysis drives the rotations of certain subunits of the enzyme against static subunits (Fig. 4).

The microchip to study the rotation of F_1-ATPase is an assembly of two PDMS chips, in which the microfluidic channels and several micropumps are embedded, and a glass chip that is coated with Ni^{2+}-nitrilotriacetic acid. F_1-ATPase is bound *via* His-tagged proteins to the glass chip. The ATP-driven rotation is observed under brightfield microscopy by enhancing the rotational motion by attaching beads. The immobilized F_1-ATPase is positioned in the centre of a microchannel that has four inlets, one of which is filled with an ATP solution; the other inlet channels contain buffer. By careful choice of flow velocities in each inlet channel, the ATP containing

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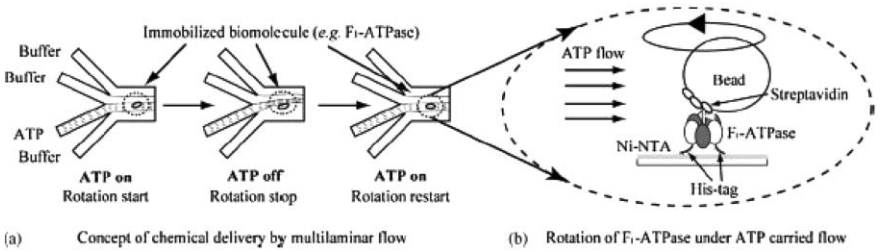

Fig. 4 The concept of single-molecule treatment by a multi-laminar chemical delivery system. (a) In the experiments described by Lee *et al.* the enzyme F_1-ATPase is immobilized in a microfluidic channel. Upon delivery of ATP, the rotational motion of the enzyme can be observed. Deflection of the ATP containing stream stops the rotation. (b) Scheme of the immobilized enzyme. A bead is attached onto the F_1-ATPase which enables visualisation of the rotational movement. (Reprinted from Lee *et al.*[4] Copyright 2006 with permission from Elsevier.)

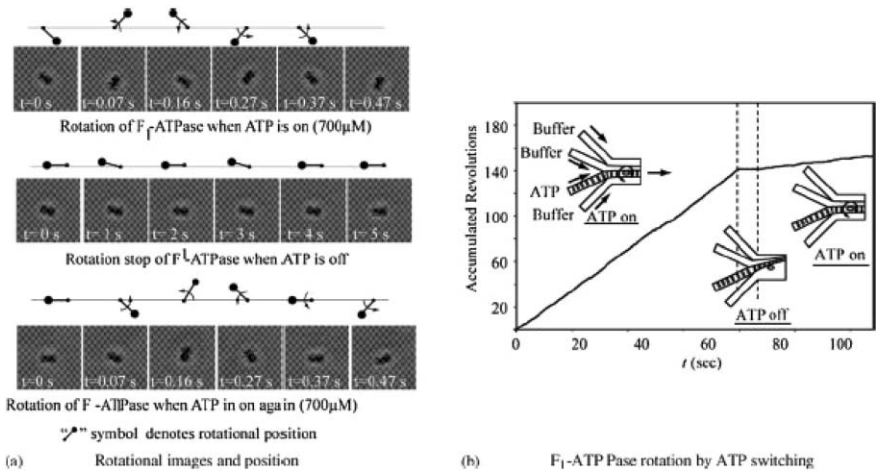

Fig. 5 Rotation of F_1-ATPase. (a) Video images showing the rotation of the bead that is attached to the F_1-ATPase. In the middle line, the ATP delivery towards F_1-ATPase is intercepted, and hence the rotation is stopped. It is restarted when ATP is supplied again to F_1-ATPase. (b) The accumulated revolution of rotation, when ATP delivery is present or stopped. (Reprinted from Lee *et al.*[4] Copyright 2006 with permission from Elsevier.)

stream is directed to the immobilized F_1-ATPase, so that rotation of the enzyme is observed. Changes in the flow velocities results in deflection of the ATP containing stream, and the F_1-ATPase rotation is stopped. In this way, the rotational motion of single F_1-ATPase enzyme can be switched on and off in a controlled manner (Fig. 5).

In this work, two discrete chemical environments were exposed to the enzyme. However, continuous gradients of chemical species could be generated, which could be a useful contribution in future to perform chemical analysis and reactions on single-molecule level.

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References

1 J.-H. Park, A. M. Derfus, E. Segal, K. S. Vecchio, S. N. Bhatia and M. J. Sailor, Local Heating of Discrete Droplets Using Magnetic Porous Silicon-Based Photonic Crystals, *J. Am. Chem. Soc.*, 2006, **128**, 7938–7946.
2 J. M. Vidic, J. Grosclaude, M.-A. Persuy, J. Aioun, R. Salesse and E. Pajot-Augy, Quantitative assessment of olfactory receptors activity in immobilized nanosomes: a novel concept for bioelectronic nose, *Lab Chip*, 2006, **6**, 1026–1032.
3 H. Kaji, M. Hashimoto and M. Nishizawa, On-Demand Patterning of Protein Matrixes Inside a Microfluidic Device, *Anal. Chem.*, 2006, DOI: 10.1021/ac060304p.
4 S. W. Lee, T. Yamamoto, H. Noji and T. Fujii, Chemical delivery microsystems for single-molecule analysis using multi-laminar continuous flow, *Enzyme Microb. Technol.*, 2006, **39**, 519–525.